# THE TAXONOMY OF *CAREX TRISPERMA* (CYPERACEAE)

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## ABSTRACT

Ora Willis Knight described *Carex trisperma* Dewey var. *billingsii* O.W. Knight in 1906, but it has not been quantitatively compared to its presumed sister taxon, *C. t.* var. *trisperma*. I tested the hypothesis implied by earlier taxonomic treatments that the two varieties are not morphologically, ecologically, geographically, and genetically distinct. To test these hypotheses, I analyzed DNA fingerprints, measured morphological characters, mapped specimen localities and recorded *in situ* canopy conditions. The two varieties are distinct based on AFLP fragment data. Based on morphological analyses, the two varieties are distinguishable by leaf width, ligule length, inflorescence length, and number of perigynia per terminal spike. *Carex t.* var. *billingsii* is a temperate plant of the northeastern United States and adjacent Canada and is confined to partially shaded/open areas in acidic bogs within deciduous forests. *Carex t.* var. *trisperma* ranges farther west, south and east than *C. t.* var. *billingsii* and grows mostly in shaded areas of bogs and swamp forests in both deciduous and boreal biomes. If *C. t.* var. *billingsii* were simply an open-grown morphotype of *C. t.* var. *trisperma*, then it would be likely that the distribution of *C. t.* var. *billingsii* would overlap that of *C. t.* var. *trisperma* and thus, be present in open bogs west of Michigan. The absence of *C. t.* var. *billingsii* in areas where *C. t.* var. *trisperma* is common suggests a genetic rather than an environmental basis for the differences in morphological characters. The geographic, ecological, morphological, and genetic data strongly suggest that *C. t.* var. *billingsii* warrants recognition at the rank of species and the new combination is effected.

KEY WORDS: *Carex*; sedge; AFLP; morphometrics; sedge ecology; phytogeography; DNA fingerprints; sphagnum bog

## RESUMEN

Ora Wills Knight describió *Carex trisperma* Dewey var. *billingsii* O.W. Knight en 1906, pero no ha sido comparado cuantitativamente con su presunto taxon hermano, *C. t.* var. *trisperma*. He probado que la hipótesis implicada en tratamientos taxonómicos previos que las dos variedades son morfológica, ecológica, geográfica, y genéticamente distintas. Para probar estas hipótesis, analicé las huellas de DNA, medí caracteres morfológicos, representé en mapas las localidades de los especimenes y tomé datos *in situ* de las condiciones del manto. Las dos variedades se distinguen en base a los datos de los fragmentos AFLP. Basándose en los análisis morfológicos, las dos variedades se pueden distinguir por la anchura de las hojas, longitud de la lígula, longitud de la inflorescencia, y número de utrículos por espiga terminal. *Carex t.* var. *billingsii* es una planta templada del Noreste de Estados Unidos y la parte adyacente de Canadá y está confinada a áreas parcialmente sombrías/abiertas en ciénagas ácidas dentro de bosques caducifolios. *Carex t.* var. *trisperma* llega hasta más al Oeste, más al Sur y al Este que *C. t.* var. *billingsii* y crece en áreas sombrías de ciénagas y pantanos forestales en biomas caducifolios y boreales. Si *C. t.* var. *billingsii* fuese simplemente un morfotipo de lugares abiertos de *C. t.* var. *trisperma*, sería probable que la distribución de *C. t.* var. *billingsii* se solapase con la de *C. t.* var. *trisperma* y estuviese presente en ciénagas abiertas del Oeste de Míchigan. La ausencia de *C. t.* var. *billingsii* en áreas donde *C. t.* var. *trisperma* es común sugiere una base genética en vez de ambiental para las diferencias en los caracteres morfológicos. Los datos geográficos, ecológicos, morfológicos, y genéticos data sugieren que *C. t.* var. *billingsii* merece el reconocimiento a nivel de especie.

During a botanical foray, in 1906, Ora Willis Knight, discovered a "peculiar little sedge" at Jewett Brook Bog in Maine. Upon further inspection, he decided that the sedge was merely a variant of typical *Carex trisperma* Dewey (Fig. 1), which was common in shaded portions of the bog. Knight named this plant *Carex trisperma* var. *billingsii* (Fig. 1) and described the foliage of *C. t.* var. *billingsii* as "setaceous or filiform," the inflorescence as having 1–2 spikelets per culm, and its habitat as "sunny." This habitat description was contrasted to that of *C. t.* var. *trisperma*, which grew "abundantly…under the trees" (Knight 1906).

The two *Carex trisperma* varieties have never been thoroughly evaluated taxonomically. Gleason and Cronquist (1991) and Toivonen (2002) briefly reported the differences between the two varieties, the most prominent of which are narrower leaves and fewer, smaller perigynia in *C. t.* var. *billingsii*. Gleason and Cronquist (1991) also reported that *C. t.* var. *billingsii* ranges from New Brunswick to Vermont and Pennsylvania. Toivonen (2002) extended that range to include Michigan and adjacent areas in Canada.

This study examines the morphology, phytogeography, ecology, and genetic relationships of the two

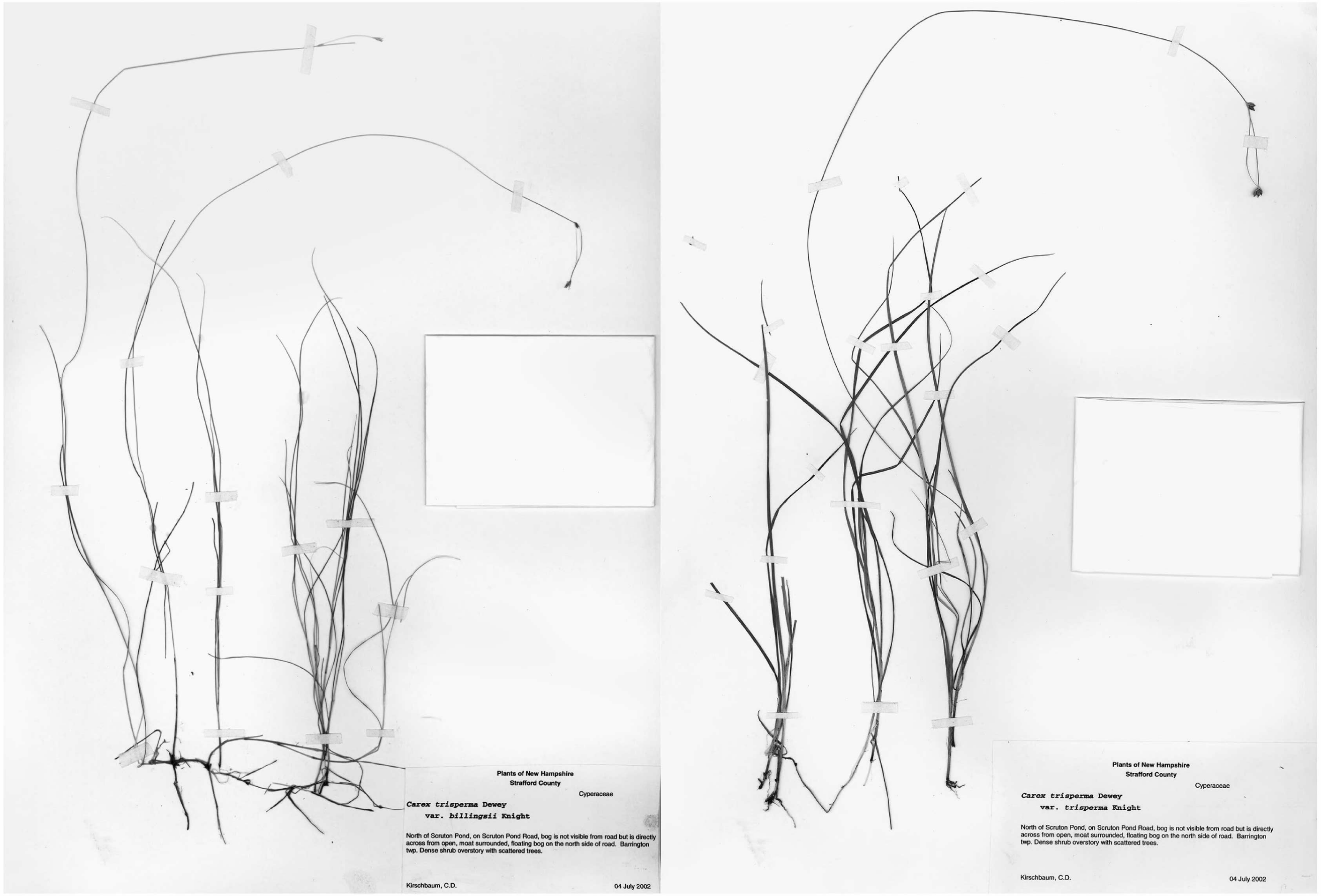


Fig. 1. The habit of *Carex trisperma* Dewey var. *trisperma* (right) and var. *billingsii* Knight (left), based on collections from New Hampshire, Strafford Co., near Scruton Pond, 04 Jul 2002, *Kirschbaum, s.n. Carex t.* var. *billingsii* is distinguished from *C. t.* var. *trisperma* with gross morphology by its narrower leaves and smaller, fewer flowered female spikes.

varieties in order to evaluate their taxonomic ranks as currently treated. I tested the hypothesis that the two varieties are distinct species using analyses of 19 morphological characters, AFLP genetic markers, habitat observations and the geographical distributions of the two taxa. If there are clear distinctions in morphology, ecology, and distribution, and if sympatric plants of both varieties can be distinguished by unique genetic markers, then raising *C. t.* var. *billingsii* to the species level will be supported. This study is the first quantitative analysis of *Carex trisperma* varieties and identifies potential variations to include in future phylogenetic studies of *Carex* sect. *Glareosae* G. Don.

## MATERIAL AND METHODS

***Field Sites and Collections Methods.—***A total of 259 individual plant specimens were collected from 22 different sites throughout the range of *C. t.* var. *billingsii* from July to September 2002 and in New York and Michigan in August and September 2003 (Table 1). The two varieties were syntopic at 13 of these sites. At two sites (Dawson Ponds and Corea Heath) *C. t.* var. *billingsii* was found without *C. t.* var. *trisperma* in the immediate vicinity of the bog (William Crins, pers. comm. and Anton A. Reznicek, pers. comm.). At all sites where both varieties were abundant, at least one individual of each variety was collected. Voucher specimens (MICH) were collected for morphological analysis, and several leaves of these specimens were immediately placed in silica gel for molecular analysis.

***Herbarium Specimens and Distribution Mapping.—***Specimens from herbaria (MICH, DAO, MT, and GH) were used for morphological measurements (along with field collected specimens) and distribution mapping. Nineteen morphological characters were selected for detailed measurement (Table 2). Data were collected from twenty specimens of each variety selected from localities throughout the Northeastern United States. Specimen label information was used to map the distributions of the two varieties A total of 310 specimens were mapped with ArcView GIS 3.2 (Environmental System Research, Inc.). Collections of *Carex trisperma* were investigated at WIS, MO, MSC, UWSP, OS and CLM to determine if *C. t.* var. *billingsii* ranged further south than previously reported. On the edge of *C. t.* var. *billingsii's* range in Michigan, sphagnum bogs in Allegan County and Barry County in Michigan's lower peninsula were also checked for the presence of *C. t.* var. *billingsii*.

Specimens of both varieties were thinly sliced with a scalpel blade to prepare specimens for scanning electron microscopy (SEM) (Jane Gillies, pers comm.). For SEM, the specimens were mounted onto a polished stub with double-stick carbon-permeated tape. As necessary, further attachment to the stubs was made with colloidal graphite. The specimens were then coated with gold and examined with an AMRay 18201 scanning electron microscope. For the anatomical cross-sectional analysis, a freezing microtone was used to obtain sections thin enough to be photographed under a compound microscope.

***Molecular analysis.—***Amplified Fragment Length Polymorphisms (AFLPs) were used to investigate species boundaries and evaluate genetic similarity within and between varieties of *Carex trisperma*. Genomic DNA was extracted from 8–12 mg of silica-dried leaf material from 12 syntopic populations and two specimens (one of each variety) from different bogs in or near Algonquin Provincial Park (APP and DLB, Table 1) for a total of 26 individuals. I isolated DNA using GenElute Plant Genomic DNA Miniprep kits (Sigma-Aldrich) with the addition of 50 units of Ribonuclease (RNase A solution, Sigma-Aldrich) to eliminate RNA contamination and 10 mg of Polyvinylpyrrolidone (PVPP, Sigma-Aldrich) to precipitate secondary compounds. RNase and PVPP were added after cell lysis and prior to incubation.

AFLP protocols followed Vos et al. (1995) with modifications by Berres (2002) and Hipp (2004). I used the selective amplification primers ("EcoRI + ATG"; 5' GAC TGC GTA CCA ATT C**AT G** 3' and "MseI + CAG";5' GAT GAG TCC TGA GTA A**CA G** 3') based on previous AFLP work in *Carex* subgenus *Vignea* section Ovales by Hipp (2006). The underlined bases on each primer correspond to the known sequences of double stranded adapters ligated to the cut ends of genomic DNA subsequent to restriction digestion. The bases in bold type are selective nucleotides employed in the AFLP process to reduce the bands amplified to an interpretable number.

TABLE 1. Collection localities of specimens used for AFLPs, morphometrics, and ecological information (*). AFLPs were screened in one individual of each variety in 12 syntopic populations except for JBB. Allopatric specimens from Ontario sites; APP and DLB were also included in the AFLP analysis. Specimen collectors include [1]AAR = Anton A. Reznicek, WJC = William J. Crins, GH = Geoffrey Hall, JH = Justin Hohn, CDK = Chad D. Kirschbaum, CJR = Carl J. Rothfels and TR = Todd Ristau (nf = not found, **at km 8 along Hwy 60).

| Site Code | Locality | State/ Province | No. of Samples variety *trisperma* | No. of Samples variety *billingsii* | Collector[1] |
|---|---|---|---|---|---|
| BPB | Bog Pond* | ME | 20 | 20 | CDK |
| CH | Corea Heath | ME | nf | 1 | AAR |
| 2ABB | Hwy 2A Bonus Bog | ME | 2 | nf | CDK |
| JBB | Jewett Brook Bog | ME | 1 | 1 | CDK |
| OPB | Otter Pond Bog* | ME | 20 | 20 | CDK |
| PMB | Petit Manan | ME | 1 | 1 | AAR |
| GLB | Gorman Lake Bog | MI | 1 | 1 | CDK + JH |
| ILB | Independence Lake Bog | MI | 1 | nf | CDK + JH |
| MNB | Minden Bog | MI | 2 | 10 | CDK |
| ML | Miner Lake Bog | MI | 1 | nf | CDK + JH |
| BHB | Bray Hill Bog* | NH | 20 | 20 | CDK |
| HPB | Heath Bog Natural Area | NH | 2 | 2 | CDK |
| MPB | Mud Pond (Fox State Forest)* | NH | 20 | 20 | CDK |
| SPB | Scruton Pond | NH | 1 | 1 | CDK |
| AB | Allenburg Bog | NY | 2 | nf | CDK + TR |
| MSL | Moss Lake Bog | NY | 1 | 1 | CDK + TR |
| LCB | Lake Carmi Bog | VT | 20 | 20 | CDK |
| QBC1 | Zone 18 | QBC | 6 | nf | GH |
| QBC2 | Zone 19 | QBC | 2 | nf | GH |
| APP | Algonquin Prov. Park** | ONT | 10 | nf | WJC |
| DLB | Dawson Ponds | ONT | nf | 1 | WJC |
| CTB | Copetown Bog | ONT | 4 | 3 | CJR |
| | | Totals | 137 | 122 | |

The final PCR product was cleaned with Centri-Sep column kits (Princeton Separations), mixed with a fluorescent-red-labeled size standard (GeneScan™ -500 ROX™, Applied Biosystems) and Hi-Dye deionized formamide (Applied Biosystems). Deantured and snap-chilled samples were loaded into a 96-well sample tray for capillary electrophoresis using ABI's Prism 310 Genetic Analyzer. The operation of the 310 Genetic Analyzer followed ABI protocols (Applied BioSystems 2000, 2001) with modifications and optimizations described in Kirschbaum (2005).

***Analytical Methods.—***I compared morphological character measurements between varieties using a two-tailed Mann-Whitney U test. Characters that were significantly different (Table 2) were included in a Principal Component Analysis (PCA) in order to assess relationships between the two taxa based on overall variation in the quantitative characters. Prior to the PCA, correlation analysis between all characters was carried out to test for character correlation, which would have heavily weighted correlated characters (Sokal & Sneath 1963; Abbott et al. 1985). None of the variables used in this analysis was significantly correlated ($p < 0.05$, $r > 0.75$) and, thus, all 19 were used in the analysis. To provide equal weight among characters, the data were Z-score transformed before conducting the PCA. These procedures standardized all measurements so that each variable had a mean of zero and a standard deviation of one (Sokal & Sneath 1963). Principal component scores for the first three axes were graphed on a scatter plot. Discriminant Function Analysis (DFA) was used to achieve maximum discrimination among samples on the basis of the transformed variables and a priori designations of samples to a taxon (i.e., *C. t.* var. *billingsii* or *C. t.* var. *trisperma*). Both PCA and

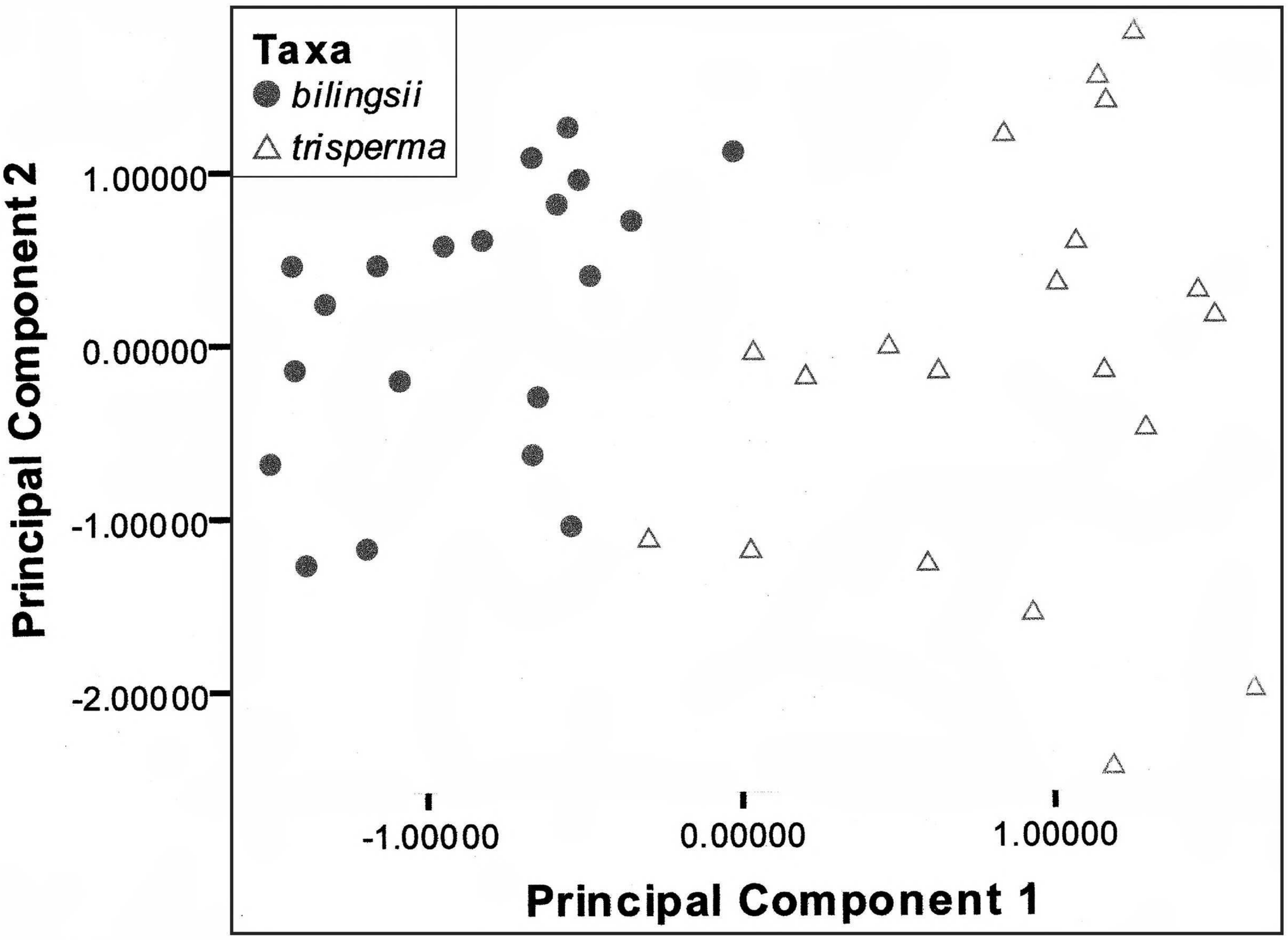


**Fig. 2. A scatter plot of the scores of Principal Components 1 and 2 for morphological characters of *Carex trisperma* Dewey varieties.**

DFA were used to determine the relative importance of characters that distinguish the two taxa. SPSS 11.5 (Statistical Package for the Social Sciences, Inc.) was used for all analyses of the morphological data.

The raw AFLP fragment data (i.e., fragment size, amplitude, etc.) were copied from ABI's GeneScan™ software to a Microsoft Excel spreadsheet. A macro was used to create a matrix of ones and zeros that denoted the presence or absence of a peak for each specimen. This matrix was then edited with reference to a GeneScan chromatograph. Small peaks that were present in at least 11 of the 13 samples from each variety were added to a plant's profile if the peak in question had an amplitude within 100 RFUs of the original amplitude threshold (800 RFU). Fragments that were present in only 1 sample out of 26 were considered to be artifacts of PCR or electrophoresis and were eliminated from the analysis. A total of 31 fragments were eliminated from the original GeneScan data set as a result of this editing.

The data matrix with the presence or absence of AFLP fragments for each specimen was used to calculate pairwise genetic distance matrices, using Nei and Li's (1979) genetic distance index for fragments in Phylip 3.62 (Felsenstein 2004) and Jaccard's (1908) distance measure in PC-ORD 4.32 (McCune and Medford 1997). Nei and Li's pairwise genetic distances were compared graphically using SYSTAT 10.2.01 (SYSTAT Software Inc.).

To examine taxon boundaries with the genetic data, I performed ordination using Nonmetric Multidimensional Scaling (NMS) in PC-ORD. NMS was chosen because, unlike phylogenetic or some phenetic approaches (e.g., UPGMA or Neighbor Joining) to analyzing molecular data, NMS does not assume that any hierarchical patterns are present in the data, an assumption that would be invalid at the start of such a study (Lessa 1990). NMS, compared to other methods of ordination, such as Principal Component Analysis, does not assume linearity among the variables and allows for the analysis of distance measures. Since Nei and

TABLE 2. Summary of quantitative characters (means, ± standard error and ranges, n=20) measured and qualitative characters observed on herbarium specimens of *Carex trisperma* varieties *trisperma* and *billingsii*. Characters that were significantly different* (two-tailed Mann-Whitney U test (MWU), asymptotic significance [*as*] < 0.05) between the two varieties were analyzed using Principal Component Analysis (PCA). Scores of the principal component (PC) axes are given for the six characters used in the PCA. **Discriminant Function Correlation Coefficient = Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions. **Highly weighted characters are highlighted.**

| Quantitative Characters | variety *trisperma* | variety *billingsii* | PC 1 | PC 2 | PC 3 | DFCC** |
|---|---|---|---|---|---|---|
| Number of spikes per inflorescence * (MWU=117.5, *as*=0.01) | 2.7±0.13 (2–4) | 2.3±0.10 (2–3) | 0.38 | 0.69 | 0.06 | -0.01 |
| Number perigynia per lateral spike * (MWU=106, *as*<0.001) | 2.9±0.2 (1–5) | 2.2±0.15 (1–3) | 0.57 | 0.17 | 0.65 | 0.03 |
| Number of perigynia per terminal spike* (MWU=59, *as*<0.001) | 3.7±0.29 (1–6) | 2.1±0.15 (1–3) | **0.71** | 0.36 | 0.30 | 0.27 |
| Achene length (mm) * (MWU=94, *as*<0.001) | 2.0±0.03 (1.7–2.2) | 1.8±0.04 (1.3–2.0) | 0.55 | 0.39 | 0.53 | 0.09 |
| Terminal Bract (Bristle) length (mm) * (MWU=124, *as*=0.04) | 51.1±2.87 (28–74) | 41.9±3.31 (15–72) | 0.56 | 0.31 | 0.15 | 0.23 |
| Inflorescence length (mm) * (MWU=51, *as*<0.001) | 38.8±2.65 (14–55) | 22.6±1.16 (14–32) | **0.78** | 0.34 | 0.12 | **0.37** |
| Ligule length (mm) * (MWU=27, *as*<0.001) | 1.1±0.08 (0.5–1.9) | 0.5±0.05 (0.3–1.2) | **0.81** | 0.20 | 0.31 | **0.41** |
| Leaf width (mm) * (MWU=0.5, *as*<0.001) | 1.2±0.06 (0.8–1.9) | 0.4±0.03 (0.3–0.8) | **0.83** | 0.25 | 0.18 | **0.78** |
| Pistillate scale width (mm) * (MWU=99.5, *as*<0.001) | 1.4±0.03 (1.1–1.7) | 1.2±0.04 (0.9–1.5) | 0.60 | 0.22 | 0.24 | 0.04 |
| Pistillate scale length (mm) (MWU=188, *as*=0.744) | 2.7±0.07 (2.2–3.2) | 2.7±0.07 (2.2–3.2) | Not analyzed in PCA " | Not analyzed in PCA " | Not analyzed in PCA " | 0.03 |
| Staminate scale length (mm) (MWU=167, *as*=0.371) | 3.2±0.13 (2.4–4.6) | 3.0±0.12 (2.2–4.3) | " " | " " | " " | 0.08 |
| Perigynia length (mm) (MWU=185.5, *as*=0.693) | 3.2±0.08 (2.2–3.7) | 3.2±0.06 (2.7–3.9) | " " | " " | " " | -0.07 |
| Perigynia width (mm) (MWU=196.5, *as*=0.923) | 1.4±0.03 (1.2–1.7) | 1.4±0.04 (0.9–1.8) | " " | " " | " " | 0.24 |
| Achene width (mm) (MWU=197, *as*=0.933) | 1.3±0.03 (1.1–1.5) | 1.2±0.04 (0.8–1.4) | " " | " " | " " | 0.31 |
| Qualitative Characters | | | " | " | " | |

Li's (1979) genetic distance index is not available in PC-ORD, Jaccard's Coefficient was used as a similarity measure. Jaccard's Coefficient has been shown to perform better than other nonevolutionary-based coefficients in elucidating relationships between closely related taxa (Landry & Lapointe 1996).

At sites in New England and Michigan, I randomly selected individuals at several bogs and noted the light conditions under which the plant was growing (either completely under a tree or shrub canopy or in a partly shaded/open condition). These two categories are based on the original habitat descriptions of the two varieties given by Knight (1906) and habitat notes from herbarium specimens. Given these broad habitat descriptions, 95% of the plants visited were precisely placed in these categories. The frequencies of 169 plants growing in each condition were analyzed by variety with $\chi^2$ analysis.

## RESULTS

***Morphology.—***The number of spikes per inflorescence, number of perigynia per lateral spike, achene

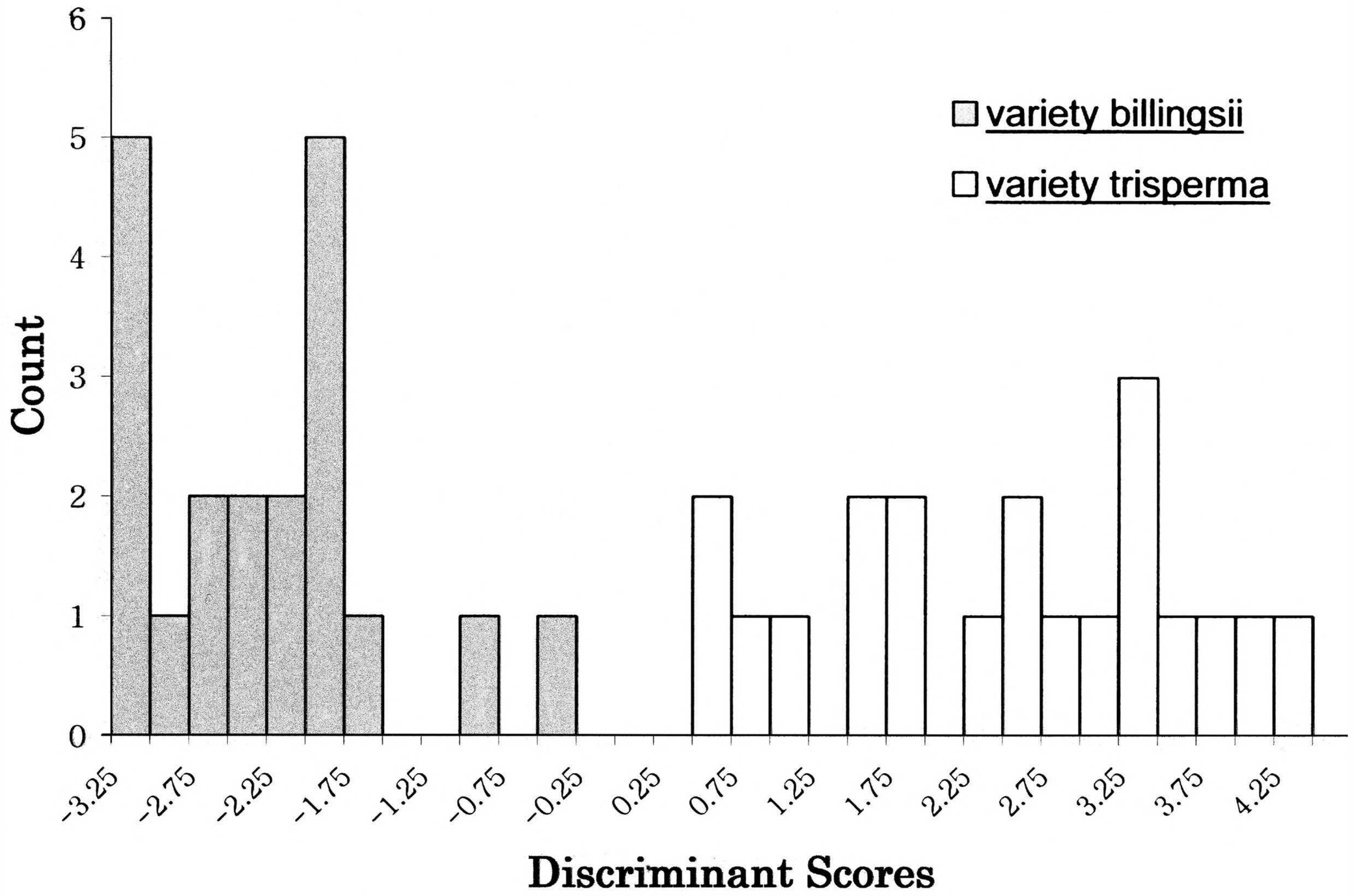


FIG. 3. A histogram of the discriminant function scores for *Carex trisperma* Dewey varieties, based on 14 measured morphological characters.

length, terminal bract (bristle) length, inflorescence length, ligule length, leaf width, and pistillate scale width differed significantly between varieties (two-tailed Mann-Whitney U test, asymp. sig. < 0.05) (Table 2). No observable differences were noted for qualitative characters, such as achene shape, sheath apex, or ligule shape and scale venation.

The overall variation of morphological characters, analyzed by PCA, is summarized by a scatter plot of the scores of Principle Components 1 and 2 (Fig. 2). These scores resolved two groups (Fig. 2). The two taxa separated on the first axis, which explained 44% of the total variance (Fig. 2). The second and third axes, however, only explained 13 and 11% of the total variance, respectively. Characters with high component (loading) scores (> 0.70) on axis one included leaf width, ligule length, inflorescence length, and number of perigynia per terminal spikelet (Table 2). As the values on axis one increases, all four of these characteristics increase and are associated with taxa described as *C. t.* var. *trisperma* (Fig. 2).

I assessed the relative importance of the morphological characters that distinguish the two taxa with Discriminant Function Analysis. A distinct separation of the two taxa is evident on a histogram of the discriminant function scores (Fig. 3). The three most highly weighted characters were the same as the highly weighted characters on Principal Component 1 in the PCA (Table 2, Fig. 6). Leaf width, ligule length, and inflorescence length will be the most useful for field recognition (Fig. 4).

The differences in leaf width originate through significant differences in leaf structure. Scanning electron microscope and leaf cross section photos demonstrate the anatomical differences that account for difference in leaf width (Fig. 5). The margins of *C. t.* var. *billingsii* leaves are involute above the sheath but fuse distally, which accounts for the triangular shape of the leaf in cross section. The leaves of *C. t.* var. *billingsii* have 1–2 large areas of aerenchyma tissue on the left and right sides of the midrib. The leaves of *C. t.* var. *trisperma* are thinly M-shaped in cross section, but appear flat apically and are deeply channeled, or keeled, on the

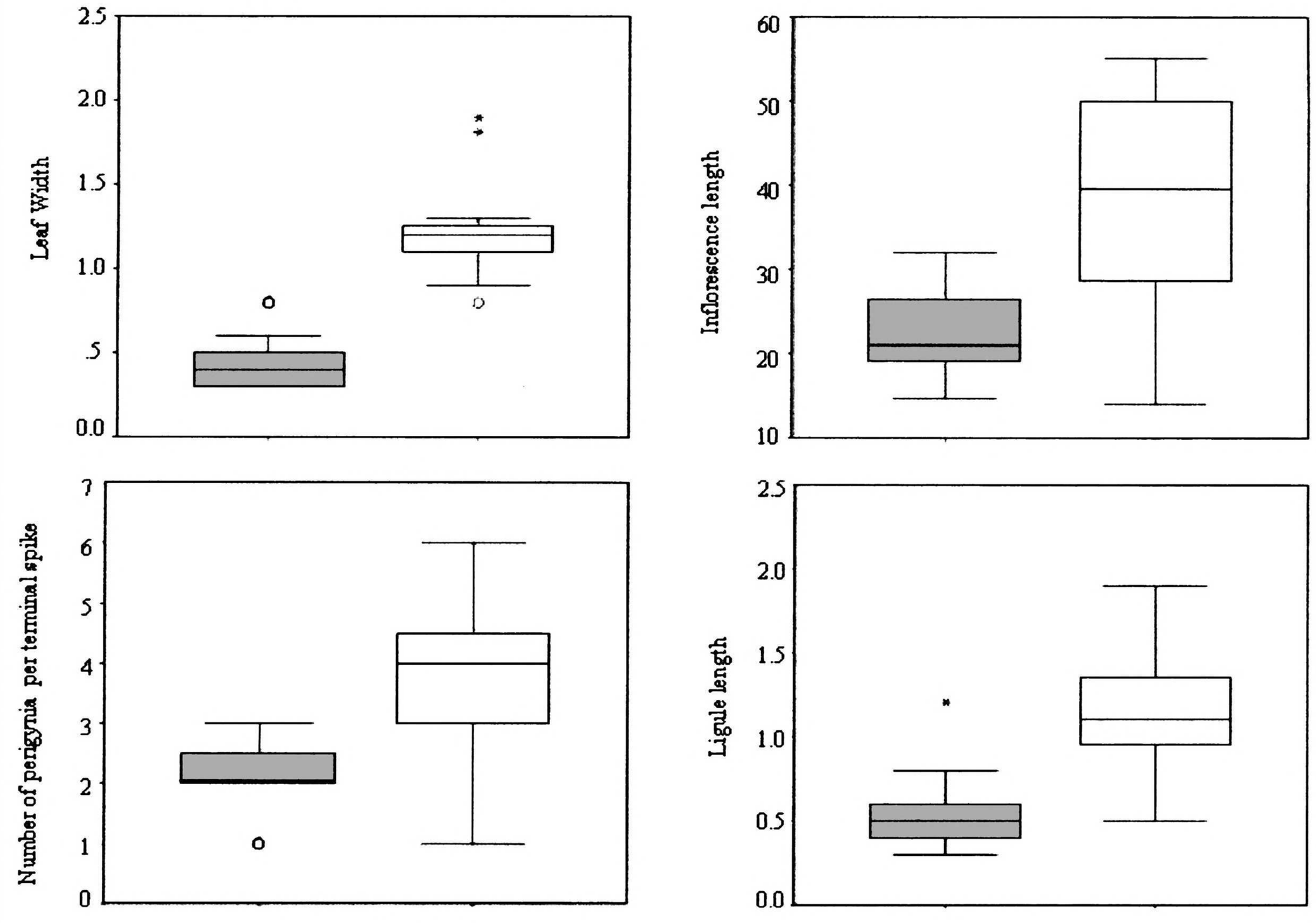


**Fig. 4. Box plots of the four morphological characters that best distinguish *Carex trisperma* varieties.**

abaxial surface, thus appearing V-shaped basally (near the sheath). There are 4–5 areas of aerenchyma tissue on either side of the midrib of *C. t.* var. *trisperma*.

***Genetic Similarity.—***A total of 102 loci were scored from GeneScan chromatographs. Ninety-two (90%) of those markers were polymorphic. DNA fragments ranged from 39 to 466 base pairs in length. The average (± standard error) fragment length was 148 ±9.4 base pairs, indicating a bias towards smaller fragments. Eighty-two markers were scored, and 80% (66) were polymorphic in *C. t.* var. *billingsii*. For *C. t.* var. *trisperma*, ninety-five markers were observed of which 86% (82) were polymorphic. With the primer pairs that were used, fragment sizes of 143, 155, and 173 base pairs were uniquely found in all specimens of *C. t.* var. *billingsii*, and fragment sizes of 154 and 178 base pairs were uniquely found in all specimens in *C. t.* var. *trisperma*.

The scatter plot of the ordination scores calculated using Jaccard's similarity index and nonmetric multidimensional scaling cleanly separated the two varieties (Fig. 6). The best solution (defined by the dimensionality with the lowest final stress from a real run) is a 3-dimensional solution with a final stress value of 13.7. All three axes explain 85% of the variance with 23%, 44%, and 18% of the va riance explained on the first, second, and third axes, respectively.

The mean intraspecific pairwise genetic distance (Nei & Li 1979) across all specimens was 0.01 (± 0.0003). Intraspecific genetic pairwise distances ranged from 0.0008 to 0.024. Genetic distances within varieties *trisperma* and *billingsii* (summarized in Fig. 7) ranged from 0.003 to 0.024 and from 0.0008 to 0.015, respectively. The mean pairwise genetic distance within var. *trisperma* was 0.011 (± 0.0004), whereas the mean pairwise genetic distance within var. *billingsii* was 0.009 (± 0.0004).

***Phytogeography.—***The two *Carex trisperma* varieties have overlapping ranges in the northeastern United

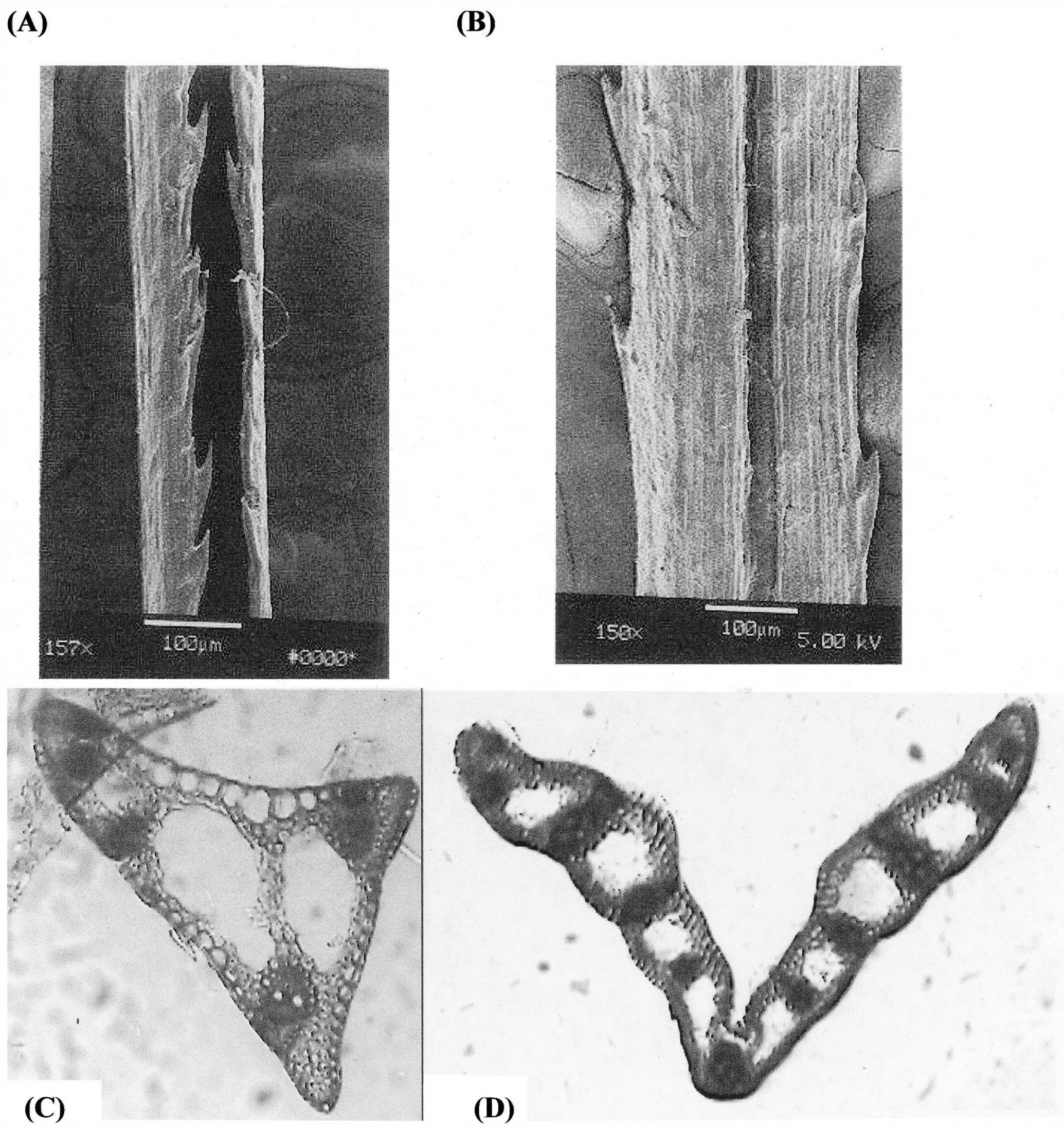


FIG. 5. Scanning electron microscope (SEM) and cross sectional photographs of leaves, which explain differences in leaf width due to internal structure. (A) SEM photograph of the basal portion of a leaf from *Carex trisperma* variety *billingsii* Knight. (B) SEM photograph of the apical portion of a leaf from *Carex trisperma* Dewey variety *trisperma*. (C) Cross sections of an apical portion of a *Carex trisperma* variety *billingsii* leaf. (D) Cross sections of a basal portion of a *Carex trisperma* variety *trisperma* leaf. (Scanning electron microscope photos were taken by Jane Gillies.)

States, southern Ontario, and Quebec (Fig. 8). Both taxa are found as far east as Newfoundland. *Carex t.* var. *billingsii* is found throughout New England, and its distribution follows the St. Lawrence seaway west through the Great Lakes. The range of *C. t.* var. *trisperma* extends farther north, south, and west. *Carex t.* var. *billingsii* ranges farther west and north than previously reported by Gleason and Cronquist (1991), but is congruent with the distribution reported by Toivonen (2002) (Fig. 8).

*Carex ttrisperma* var. *billingsii* may be more widespread than previously described. The farthest southwestern record of *C. t.* var. *billingsii* is from Ingham County, Michigan (Parmelee 246 MSC, 134054). A specimen of *C. t.* var. *billingsii* collected in Wexford County, Michigan (south of Traverse City), in the northern

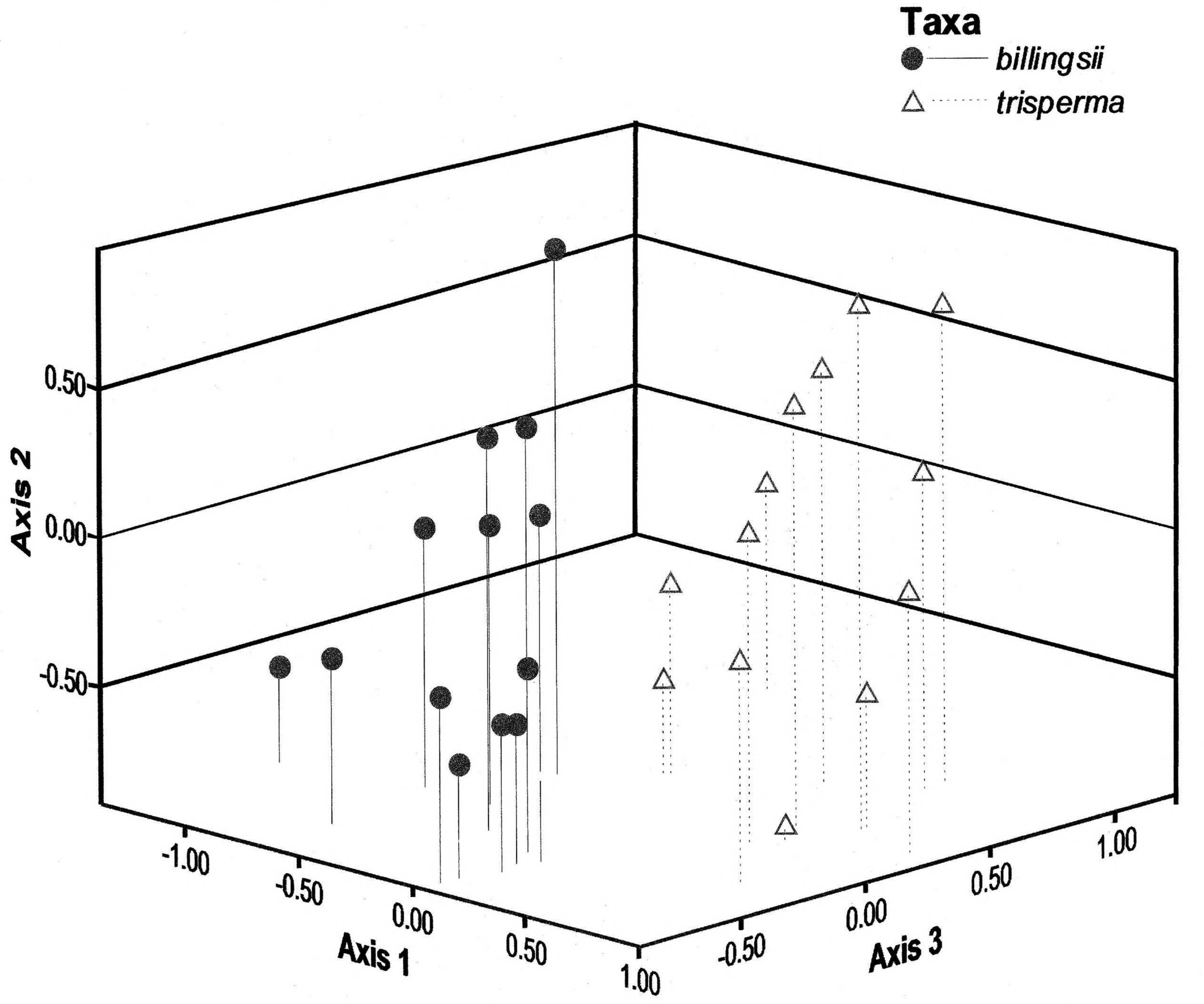


**Fig. 6. A 3-dimensional scatter plot of nonmetric multidimensional scaling ordination scores for AFLP marker data derived from Carex trisperma Dewey varieties *billingsii* Knight and *trisperma*.**

Lower Peninsula, recently found at MICH, expands the taxon's range farther north in Michigan. Searches in southwestern lower Michigan, however, did not disclose any further localitities. Specimens recently collected from Allegany County in New York (*Kirschbaum, s.n.*) and Susquehanna County in southeastern Pennsylvania (Naczi 10065.) suggest potential localities farther inland and away from coastal areas of the eastern seaboard and Great Lakes. Ombrotrophic bogs in northwestern Pennslyvania, northern Ohio, and Indiana are potential locations for new populations of *C. t.* var. *billingsii*. However, specimens reviewed at WIS, MO, UWSP, OS, and CLM yielded no further range extensions for *C. t.* var. *billingsii*.

***Ecology.—****Carex t.* var. *billingsii* mostly grows in full-to-partial sunlight. Often it grows in dense clumps in ombrotrophic bogs at the base of low-growing ericaceous shrubs or along deer trails and narrow water channels. Unlike *C. t.* var. *billingsii*, *C. t.* var. *trisperma* is not restricted to acidic sphagnum bogs and, on the basis of herbarium label data, is commonly found in densely shaded swamp forests of various floristic, hydrological, and edaphic compositions. At many of the sites, I also found *C. t.* var. *trisperma* in swamps that were deeply shaded by tree canopies that were adjacent to the main bog mat. When growing in bogs *C. t.* var. *trisperma* is commonly (but not always) found in shaded portions such as dense tamarack or ericaceous shrubs stands.

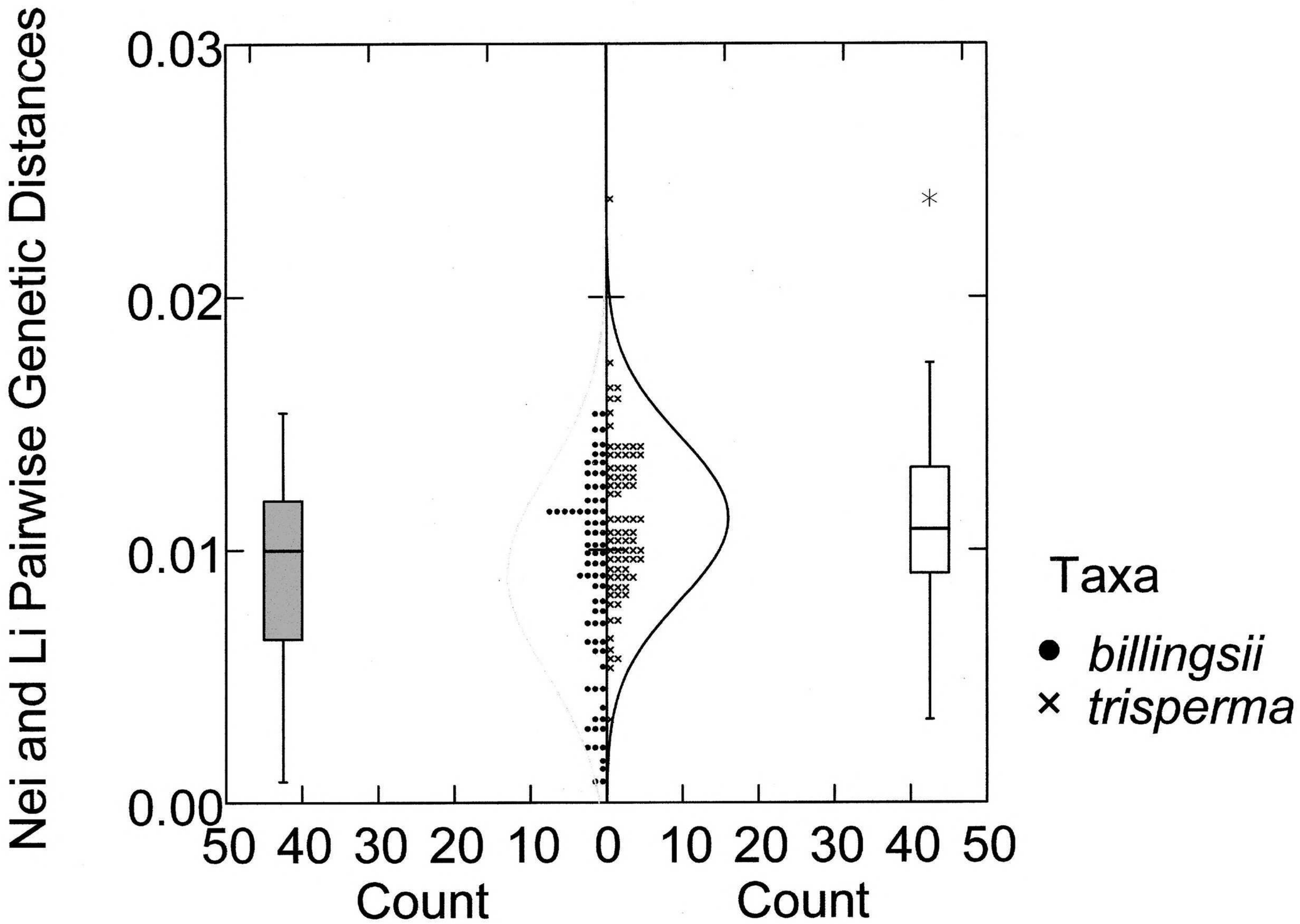


**Fig. 7. A summary of Nei and Li pairwise genetic distances between specimens, which were calculated from the presence or absence of AFLP fragments.**

Several individuals of *C. t.* var. *trisperma* were documented in this study and on herbarium labels as growing in full sunlight, and several *C. t.* var. *billingsii* were noted as growing in shaded areas. These individuals retain their expected morphological characteristics despite growing in a-typical habitats. Seventy-six percent of the 89 *C. t.* var. *billingsii* observations I collected were growing in open and slightly shaded (from low-growing shrubs) areas and 24% of the specimens were found under tall shrub or tree canopies. Sixty-four percent of the 80 *C. t.* var. *trisperma* observations I collected were found growing under tall shrub or tree canopies and 36% were growing in open and slightly shaded conditions. The proportion of plants growing in the predicted, "typical" light conditions for *C. t.* var. *trisperma* and *C. t.* var. *billingsii* was significantly different ($\chi^2 = 5.7$, $p < 0.02$ and $\chi^2 = 21.1$, $p < 0.001$, respectively) from expected proportions of 50% of the specimens in each condition.

## DISCUSSION

***Morphology.—***PCA and DFA support the recognition of two distinct entities among the herbarium specimens measured in this study (Figs. 4, 5, and 6). Both multivariate analyses place high importance on leaf width, ligule length, inflorescence length, and the number of perigynia per terminal inflorescence in discerning the two varieties (Table 2, Fig. 6). The more diminutive measurements of *C. t.* var. *billingsii* agree with previous descriptions by Knight (1906), Gleason and Cronquist (1991), and Toivonen (2002). The lack of morphologically intermediate specimens and the clear morphological distinctions between the two taxa, based on analysis of several characters simultaneously (Fig. 4), do not support Knight's contention that *C. t.* var. *billingsii* is a transitional form of *C. t.* var. *trisperma*.

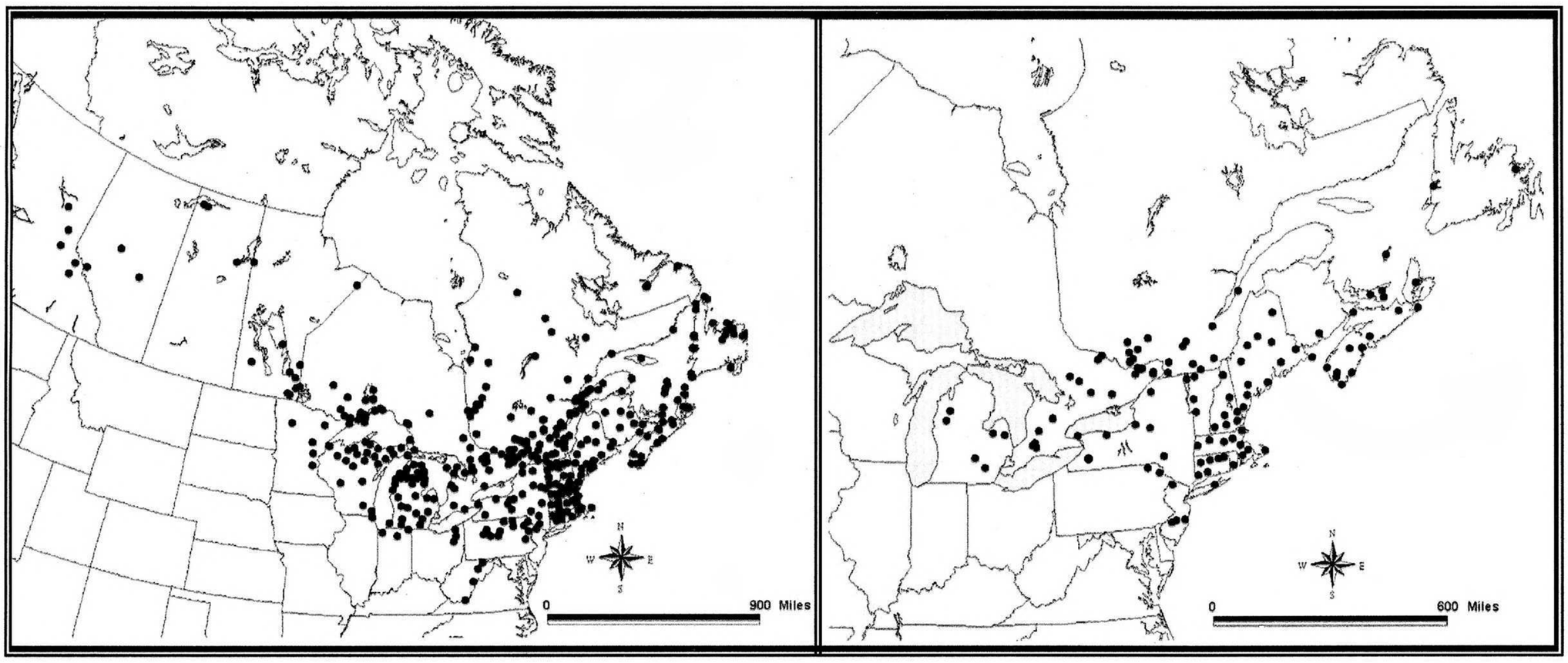


**Fig. 8. The distribution of *Carex trisperma* Dewey varieties *trisperma* (left) and *billingsii* Knight (right) mapped from 310 herbarium specimens and recent collections.**

***Phytogeography and Ecology.*—***Carex trisperma* var. *billingsii* and var. *trisperma* each have a unique geographic distribution and ecology (Figs. 2–3). *Carex t.* var. *billingsii* has been found only in the northeastern United States and adjacent Canada. Despite occurring in *Sphagnum* bogs, *C. t.* var. *billingsii* is a temperate plant and is mostly confined to sites within the deciduous forest biome (Brown & Lomolino 1998). The distribution of *C. t.* var. *trisperma*, however, spans the deciduous forest biome and reaches into the boreal or sub-arctic biome (Brown & Lomolino 1998) and extends farther west, south, and north than that of *C. t.* var. *billingsii*.

In many sites in the east, the two varieties are syntopic (Table 1); however, *C. t.* var. *billingsii* has never been collected west of Michigan. If *C. t.* var. *billingsii* were simply an open-grown morphotype of *C. t.* var. *trisperma*, then it would be likely that the distribution of *C. t.* var. *billingsii* would overlap that of *C. t.* var. *trisperma* and thus be present in open bogs west of Michigan. However, *C. t.* var. *billingsii* has not been collected in any of the well-studied bogs of southern Wisconsin or northern Minnesota, regions known for peat bogs that are found in glacial lake beds (i.e., Glacial Lake Wisconsin and Agassiz Lake). Thus, the absence of *C. t.* var. *billingsii* in areas where *C. t.* var. *trisperma* is common suggests a genetic rather than an environmental basis for the differences in morphological characters.

Comparisons of the canopy conditions in which the two varieties were found provide evidence of some ecological distinction between the two taxa. *Carex t.* var. *trisperma* is typically found in shaded conditions, and *C. t.* var. *billingsii* is typically found in open conditions. This lack of morphological plasticity in opposing light conditions was also noted on herbarium specimens. For example, a specimen of *C. t.* var. *trisperma* collected by Reznicek, 11367, Naczi and Case was "from a small colony in full sun on a hummock in the open bog." Each taxon retains its morphological characters across habitats with different light conditions.

These observations agree with Anderson et al. (1996), who quantified habitat characteristics of *C. trisperma* in Maine. Even though they did not analyze their data by variety, they found that *Carex trisperma* grew under a range of shaded-to-open conditions with a mean percent canopy cover of 53 (± 35.8 S.D.).

**Genetic Analyses.—**Nei and Li's (1979) genetic distances within varieties *trisperma* and *billingsii* ranged from 0.003 to 0.024 and 0.0008 to 0.015, respectively (Fig. 8). Hipp (2004), in a study of the *Carex tenera* Dewey group of section *Ovales* Kunth, provided the only reasonable benchmark for comparison within *Carex*. Genetic distances ranged from 0.03 to 0.08 among three species in the *C. tenera* group (Hipp 2004). These numbers indicate a much higher level of genetic divergence between species in the *C. tenera* group

than between the *C. t.* varieties. However, the species of the *Carex tenera* group can be diagnosed easily with morphological characters (Hipp 2004), as can the varieties of *Carex trisperma* (Figs. 4–6). This suggests that in *Carex*, morphological distinctiveness does not always correlate with genetic divergence.

Nonmetric multidimensional scaling supports genetic distinction between the two varieties. Intravarietal genetic distances were significantly higher in *C. t.* var. *trisperma* than in *C. t.* var. *billingsii*. This suggests greater genetic diversity in *C. t.* var. *trisperma*, which is also consistent with its relatively more extensive distribution compared to that of *C. t.* var. *billingsii*.

While genetic differentiation alone is not sufficient for delimiting species boundaries, the molecular analyses combined with the consistent differences in morphological, geographical, and ecological data to strongly suggest that *C. t.* var. *billingsii* is distinct enough from *C. t.* var. *trisperma* to warrant raising *C. t.* var. *billingsii* to the rank of species. Indeed, phenotypic clusters based on morphological data in plants often correspond closely to independent reproductive lineages identified by crossing studies (Rieseberg et al. 2007). The fact that morphometric data and molecular genetic data both demonstrate strong differences between the varieties tested in this study supports raising these varieties to species rank.

## CONCLUSION

The morphological data presented in this paper suggest that *Carex trisperma* and *Carex billingsii* comb. et stat. nov can be separated on the basis of a distinct set of morphological characters, of which leaf width, ligule length, inflorescence length, and the number of perigynia are the most diagnostic. Based on phenetic analyses of the AFLP fragment data using nonmetric multidimensional scaling, the two varieties are distinct. The combination of geographic, ecological, morphological, and genetic data strongly suggest that *Carex trisperma* var. *billingsii* is distinct enough from *C. t.* var. *trisperma* to warrant raising *C. t.* var. *billingsii* to the rank of species.

Future phylogenic analyses that evaluate evolutionary relationships within sect. *Glareosae* should include these two species along with other putative members of this group. The author hopes that the recognition of *C. billingsii* at the rank of species will bring this species to the attention of wetland scientists and conservation biologists who can further study the ecological differences between it and *Carex trisperma* and also further document the abundance of *C. billingsii* in bogs on the fringe of its distribution to determine whether protection status is warranted.

## TAXONOMIC TREATMENT AND KEY TO SPECIES

Leaves 0.8–1.9 mm wide, flat or thinly M–shaped, ligules 0.5–1.9 mm long, inflorescences (14–)23–55 mm long, spikes per inflorescence (2–)3–4, terminal spikes with (1–)2–6 perigynia per spike _____**Carex trisperma**

Leaves 0.3–0.8 mm wide, filiform-involute, ligules 0.3–0.8(–1.2) mm long, inflorescences 14–32 mm long, spikes per inflorescence 2–3, terminal spikes with 1–3 perigynia per spike_____**Carex billingsii**

## NOMENCLATURE

**Carex billingsii** (O.W. Knight) C.D. Kirschbaum, comb. et stat. nov. BASIONYM: *Carex trisperma* var. *billingsii* O.W. Knight, Rhodora 8:185. 1906. *Carex trisperma* f. *billingsii* (O.W. Knight) B. Boiven, Naturaliste Canad. 94:523. 1967. TYPE: U.S.A. MAINE: Somerset Co.: Pleasant Ridge Twp., the drier portions of upper Jewett Bog, 5 Jul 1906, *O.W. Knight, J. Murdoch Jr., E.B. Chamberlain, R.A. Ware, S. Rollins 5066* (HOLOTYPE: not seen, see below; ISOTYPE: GH!).

It is not certain where Knight's holotype for *C. t.* var. *billingsii* was deposited. I have contacted curators at MAINE, VT and GH but they were not able to locate the type specimen. A duplicate of the type (cited above) is located at GH and another specimen, *Knight 2021*, collected at the same locality and on the same date as the specimen that Knight cited as the type specimen is also at GH.

One untypified name referable to the *Carex trisperma* alliance exists, *Carex quaternaria* Sprengel (1826), based on material from New Jersey. The type of *Carex quaternaria* appears to have been destroyed at Berlin. However, the description states "Spiculis 4 floris" (spikes four-flowered) (Sprengel 1826) (presumably the basis for the specific epithet *quarternaria*). *Carex billingsii* is smaller and averages fewer flowered spikes than *C. trisperma*, and no specimen has been observed with four perigynia in a spike. *Carex quaternaria* is cer-

tainly the same entity as *Carex trisperma*, which can have up to six perigynia per spike. A neotype for *Carex quaternaria* from New Jersey and having some spikes with 4 perigynia is selected below.

***Species Description.—****Carex billingsii*. Plants glabrous, loosely cespitose; rhizomes with slightly fibrous or non-fibrous sheaths, basal sheaths dark to light brown. Fertile culms erect at anthesis, elongating and arching toward the ground after fruiting, 20–36 cm with 2–3 spikes per inflorescence. Leaves of the fertile culm (1)3 or 4 located on the lower 1/3 of the culm. Leaf blades filiform-involute above the sheath, leaf margins fusing distally, 7–13 × 0.3–0.8 mm. Leaves triangular in cross section, with 1–2 large areas of aerenchyma tissue on the left and right sides of the midrib. Ligule 0.3–0.8(–1.2) mm long, obovate or with rounded apex. Sheaths hyaline, apex concave. Inflorescence 14–32 mm long, proximal bract 15–63(–72) mm, often exceeding inflorescences. Base of proximal bract sometimes elongated and expanded 1.7–3.2(–4.1) × 0.8–1 mm. Terminal spikes 3.3–4.7 × 1.9–3.2 mm with 1–3 perigynia per spike, lateral spikes 3.5–4.5 × 1.5–2.5(–3.1) mm with 1–3 perigynia per spike. Spike with 2 staminate flowers and ascending to slightly diverging perigynia. Proximal-most and penultimate spikes 2.2–6 mm apart, penultimate and distal-most spike (when present) 6–23 mm apart. Perigynia 2.7–3.9 × 0.9–1.6(–1.8) mm widest just below the middle. Beak of the perigynia 0.4–0.7 mm, truncate to bi-dentulate with teeth 0.1 mm long. Abaxial nerves of the perigynia 14–19(–25), adaxial nerves 9–12. Pistillate scales 2.2–3.2 × 0.9–1.5 mm with green midrib between whitish-green margins, apex acute (mucronate), 1/2–2/3 as long as the perigynia. Stigmas 0.03–0.1 mm wide. Staminate scales 2.2–4.3 × 0.9–1.4 mm with similar coloring as pistillate scales. Anthers 0.9–1.5 mm long. Achenes 1.3–2.0 × 0.8–1.4 mm, ovate, broadly elliptical or obspatulate. Base of the achene rounded, truncate, cuneate or attenuate, apex rounded or truncate. Fruiting mid-June–September. Found in sunny and in shaded areas of ombrotrophic, tamarack, spruce and cedar bogs, often on tops and sides of sphagnum hummocks, along trails and small creeks, wet, sandy swales in NJ pine barrens, *Chamaecyparis* Spach swamps bordering salt marshes in MA and interdunal string bogs in Quebec. Often associated with ericaceous shrubs such as *Kalmia angustifolia* L., *Chamaedaphne* Moench, *Ledum* L. and *Vaccinium* L.

Specimens examined: **CANADA. NEWFOUNDLAND:** Trepassey, Avalon Peninsula, 053°24'00"W, 46°43'00"N, 16 Aug 1924, *Fernald, Long, Dunbar 26376* (GH). **ONTARIO:** 6 Jul 1915, *Frere Rolland-Germain s.n.* (DAO); 12 Jul 1923, *Malte s.n.* (MICH); Middlesex, Sifton (Byron) Bog, UTM Grid 734575, Map 40 I/14, 081°25'00"W, 43°00'00"N, 4 Jul 1991, *Reznicek, McLeod 8810* (MICH). **PRINCE EDWARD ISLAND:** Kings, Murray River, N of the river on Route 24, 062°32'00"W, 46°02'00"N, 22 Jul 1953, *Erskine, Smith 2103* (DAO). **QUEBEC:** Berthier, Lanoraie, 073°13'00"W, 45°58'00"N, 6 Jul 1932, F. Marie-Victorin, *F. Rolland-Germain 49208* (MT); Kamouraska, Peat bog 1 mi NE of Riviere Ouelle, 069°49'00"W, 47°32'00"N, 6 Aug 1947, *Calder 1282* (DAO); Labelle, NE du Grand lac Nominingue, pres de la riviere Rouge, Bellerive, 27 Jul 1939, *F. Lucien, F. Eloi 577* (MT); North Bay, 3 mi N of Tomiko River bridge along Hwy 11, N of North Bay, 23 Sep 1959, *Calder, Kukkonen 24287* (DAO); Gatineau, Masham Twp., Conc. V, (Outaouais), 13 km WNW of Wakefield, 31 F/9 156575 (UTM grid), Ottawa District, 076°05'W, 45°40'N, 29 Jun 1988, *Reddoch 398* (DAO); Les Sillons, W side of Hwy 199, S of Pont du Detroit, N end of Ile du Hvre aux Maisons, 14 Aug 1998, *Oldham 21198* (MICH).

**UNITED STATES. CONNECTICUT. Hartford Co.:** Burlington, 11 Jun 1921, *Weatherby D2120* (GH). **Hartford Co.:** Black Spruce bog S of Rte 168 near Congamond Lake, 22 Jun 1982, *Mehrhoff 6343* (MICH). **Litchfield Co.:** Kent, 4 Jul 1930, *Torrey s.n.* (MICH). **Tolland Co.:** bog near railroad tracks and Rte I-84, 4 Jul 1990, *Mehrhoff 13514* (MICH). **MAINE. Aroostook Co.:** T8N, R5W, 25 Jul 1941, *Pease, Bean 29029* (GH); Fort Fairfield, the Aroostook River Basin, 5 Jul 1940, *Chamberlain 1715* (GH); E side of Hwy 2A (Alt 2) ca. 2.7 mi S of Forkston-TAR2WELS township line. 0577721E 5082451N UTM Zone 19T (Units: km, Datum: NAD 83), 6 Jul 2002, *Kirschbaum s.n.* (n/a). **Hancock Co.:** Corea Heath, SW side of Hwy 195 ca 1 mi NW of Corea, 068°00'W, 44°23'N, 16 Jul 1992, *Reznicek 9149* (MICH); head of Torrey Pond, Deer Isle, 7 Jul 1915, *Hill 2179*, (GH). **Somerset Co.:** Jewett Brook Bog, NE of Jewett Pond, 150 ft NW of Jewett Brook and past the turn to Jewett Pond campsites, 4.7 mi NW of Cross rd. along Rowe Pond Rd., Pleasant Ridge Twp. 0420195E 4998596N UTM Zone 19T (Units: km, Datum: NAD 83), 7 Jul 2002, *Kirschbaum s.n.* (n/a). **Washington Co.:** 8 Jul 1993, *Reznicek 9620*, (MICH). **MASSACHUSETTS. Barnstable Co.:** Harwich, 3 Aug 1913, *Weatherby s.n.* (GH). **Berkshire Co.:** Lost Pond, Becket, 16 Jul 1909, *Hoffmann s.n.* (GH). **Middlesex Co.:** Littleton, No date, *Manning(?) s.n.* (GH). **Middlesex Co.:** Tewksbury, no date, *Gray(?) s.n.* (GH). **MICHIGAN. Washtenaw Co.:** Gorman Lake Bog on the S end of Gorman Lake off Lindley Rd. in Waterloo State Recreation Area, ca. 8 mi NW of Dexter., 21 Sep 2002, *Kirschbaum s.n.* (n/a). **NEW HAMPSHIRE. Cumberland Co.:** Bog Pond, 0.75 km off Haskell Hill Rd. by way of power line right-of-way on private property. Northern Harrison twp., 0370429E 4886298N UTM Zone 19T (Units: km, Datum: NAD 83), 7 Jul 2002, *Kirschbaum s.n.* (n/a). **Hillsborough Co.:** off hiking trail on the W side of Mud Pond in Fox State Forest. Hillsborough twp., 0264272E 4780594N UTM Zone 19T (Units: km, Datum: NAD 83), 4 Jul 2002, *Kirschbaum s.n.* (n/a). **Strafford Co.:** between Mt. Hussey and Mt. Chesley, Farmington, 20 Jul 1967, *Hodgon 15735* (GH); N of Scruton Pond, on Scruton Pond Rd., Barrington twp., 4 Jul 2002, *Kirschbaum s.n.* (n/a). **NEW JERSEY. Burlington Co.:** Chatsworth, 1 Jul 1932, *Hermann 3380*, (MICH). **Ocean Co.:** Forked River, No date, *Churchill s.n.* (GH); Pole Bridge Brook, 2 mi WSW, Whitings, 17 Jul 1914, *Long 10321* (GH). **NEW YORK. Allegany Co.:** Moss

Lake Bog on Moss Lake off Sand Hill Rd. ca. 2.5–0.0 mi SW of Houghton, Caneadea twp. 0731623E 469767N UTM Zone 19T (Units: km, Datum: NAD 83), 11 Jul 2002, *Kirschbaum s.n.* (n/a). **PENNSYVANIA. Susquehaanna Co.:** 2 mi N of Burnwood, W side of Ball Lake, 24 Aug 2003, *Robert F.C. Naczi 10065* (DOV). **VERMONT. Franklin Co.:** 055°45'00"W, 51°33'00"N, 25 Jul 1912, *Woodward s.n.* (GH). **Grand Isle Co.:** S. Alburg, 16 Jul 1939, *Knowlton s.n.* (GH).

**Carex trisperma** Dewey, Amer. J. Sci. Arts 9:63. 1825. Basionym: *Neskiza trisperma* (Dewey) Raf., Good Book. Amenit. Nat. Philad. 27. 1840. Type: U.S.A. Massachusetts: Berkshire Co.: Williamstown, without collection date, *C. Dewey* (lectotype designated here: GH 63033).

U.S.A. Massachusetts. [probably Bershire Co.]: Williamstown-Deerfield, grows in the form of bogs in sphagnous place among hills, [no collection date], *C. Dewey* (GH 27464).

*Carex quaternaria* Spreng., Systema Vegetabilium 3:809. 1826. Type: U.S.A. New Jersey. Sussex Co.: Culvers Gap, swamp, 30 May 1919, *Ludlow Griscom, No. 14336* (neotype designated here: GH-s.n.)

**Species Description.** *Carex trisperma*. Plants glabrous, loosely cespitose; rhizomes with slightly fibrous or non-fibrous sheaths, basal sheaths dark to light brown. Fertile culms erect at anthesis, elongating and arching toward the ground after fruiting, 15–65 cm with (2–)3–4 spikes per inflorescence. Leaves of the fertile culm (2)3 or 4(5) located on the lower 1/3 of the culm. Leaf blades, appearing flat apically and deeply channeled, or keeled on the abaxial surface, (9–)13–18(–24) × 0.8–1.9 mm. Leaves thinly M-shaped in cross section with 4–5 areas of aerenchyma tissue on each side of the midrib. Ligule 0.5–1.9 mm long, obovate or with rounded apex. Sheaths hyaline with sheath apex concave. Inflorescence (14–)23–55 mm long, proximal bract 28–74 mm, often exceeding inflorescences. Base of proximal bract sometimes elongated and expanded 1.5–3.7 × 0.6–14 mm. Terminal spikes 4.4–6.5 × 2.5–4.4 mm with (1–)2 perigynia per spike, lateral spikes 3.6–5.0 × 2.4–4.7 mm, with (1–)2–6 perigynia per spike. Spike with 2 staminate flowers and ascending to slightly diverging perigynia. Proximal-most and penultimate spikes 4.3–12 mm apart, penultimate and distal-most spike (when 3 spikes are present) (14)21–33(46) mm apart. Perigynia 2.2–3.7 × 1.2–1.7 mm widest just below the middle. Beak of the perigynia 0.4–0.7 mm, truncate to bi-dentulate with teeth 0.1–0.2 mm long. Abaxial nerves of the perigynia 13–21, adaxial nerves 7–14. Pistillate scales 2.2–3.2 × 1.1–1.7 mm with green midrib between whitish-green margins, apex acute to mucronate, 1/3–2/3 as long as the perigynia. Stigmas 0.05–0.1 mm wide. Staminate scales 2.4–4.6 × 0.5–1.2 mm with similar coloring as pistillate scales. Anthers 1–1.5 mm long. Achenes 1.7–2.2 × 1.1–1.5 mm, ovate, broadly (narrowly) elliptical or obspatulate. Base of the achene cuneate or attenuate, apex rounded or truncate. Fruiting early June–August. Found in deep to partial shade and occasionally in sunny areas of ombrotrophic sphagnum bogs and cedar and spruce swamp forests and wet-mesic deciduous woods. Associated with *Picea rubens* Sarg., *Abies balsamea* (L.) P. Mill., *Taxus canadensis* Marsh., *Acer rubrum* L. and *Pinus strobus* L. in bogs and swamps of New England, *Chamaecyparis* Spach swamps in MA, *Thuja occidentalis* L., *Picea mariana* (P. Mill.) B.S.P., *Acer rubrum* L. and *Larix* P. Mill. swamp forests from New York and Ontario westward and open Sphagnum-Ericaceae bogs across its range, especially northward.

Specimens examined: **CANADA. ALBERTA:** Swan Hills Twp., 65–R 9–W5M, 115°45'00"W, 54°45'00"N, 7 Aug 1960, *Pegg 880* (DAO). **BRITISH COLUMBIA:** Barkerville, 12.5 mi by road NNE of Barkerville on road to Bowron Lake, 121°23'00"W, 54°14'00"N, 9 Aug 1954, *Calder, Savile, Ferguson 14328* (DAO). **NEW FOUNDLAND:** Virginia Water, near St. John's, No date, Robinson, *Schrenk 100* (GH); near Isthmus Cove, Pistolet Bay, 5 Aug 1925, Wiegand, *Gilbert, Hotchkiss 27612* (GH); Forteau, Belle Isle, 14 Aug 1925, *Long 27614* (GH); Goose Bay, 060°21'W, 53°19'N, 10–12 Aug 1949, *Schofield 749* (DAO). **MANITOBA:** Lac Du Bonntet, 7 Jul 1949, *Breitung 7474* (DAO); Lac du Bois, along trail to South Lake, 095°40'W50°16'N, 18 Aug 1982, *Keleher 954* (DAO); near Keyhole Lake, inland from NW shor of Tod Lake., 101°45'50"W56°34'06"N, 20294, 24 Jul 1955, *Ritchie 1268* (DAO); NW side of Tulibi Lake, 19 Jun 1955, *Ritchie 822* (DAO); Taiga Biological Station, Wallace Lake., 095°20'W51°02'N, 5 Jul 1979, *Keleher 737* (DAO). **NOVA SCOTIA:** Antigonish, vicinity of Seascape Cottages, W of Bayfield, 062°00'00"W, 45°37'00"N, 20 Jun 1992, *Oldham 13841* (MICH). **ONTARIO. Nipissing District:** 0.25 mi S of Jack Lake on old logging road, 078°33'00"W, 45°35'00"N, 12 Jun 1958, *Kazdan 617* (DAO); Mowe L. Rd just S. of Plummes L. on recently built road shoulder, 090°43'W, 48°20'N, 27 Jun 1981, *Garton 20180* (MICH). **Norfolk Regional Municipality:** Turkey Point Provincial Park, Wilderness Zone, Delhi Tp. Mun., UTM 545265 (40I/9) (sq17NT52), 080°19'00"W, 42°42'00"N, 27 May 1987, *Oldham, Sutherland, Kirk 7206* (MICH); Bruce, Schmidt L, 29 Jul 1987, *Johnson s.n.* (MICH). **SASKATCHEWAN:** S shore of Lake Athabasca, E of William River. Vicinity of "Little Gull" Lake., 109°00'W59°01'N, 22826, 29 Jun 1962, *Angus 295-62* (DAO); ca. 5 km SSE of Archibald Lake, ca. 13 km NNW of Davy Lake, 108°30'W58°58'N, 29078, 8 Jul 1979, *Harms, 27411* (DAO); Wollaston Lake Rd, mile 10, Hwy 105,

ca. 130 mi N of La Ronge., 103°37'W56°20'N, 22 Jul 1973, Ternier *s.n.* (DAO). **QUEBEC:** 075°55'51"W, 45°38'18"N, 12 Jul 1922, *Malte 384/22* (MICH); Rupert House, E coast of James Bay, 18 Jul 1929, *Potter 91* (GH); Red Bay, N shore of the Gulf of St. Lawrence, 26 Jul 1929, *Abbe 1062* (GH); Grand Lake, Blue Grass Brook, near Camp 11, 5 Aug 1951, *Rolueau 2206* (MICH); Abiti-East, Harricanaw River Maizerets Twp. 0.75 mi E of river, 078°03'W, 49°11'N, 24 Jul 1958, *Bentley 58163* (DAO).

**UNITED STATES. CONNECTICUT. Windham** Co.**:** Windham, 17 Jun 1914, *Weatherby 3472* (GH). **ILLINOIS. Lake Co,:** 3 Aug 1906, Gleason, *Shobe s.n.* (MICH). **MASSACHUSETTS. Plymouth Co.:** Norwell, 6 Jun 1932, *Knowlton s.n.* (GH). **Worcester Co.:** 17 Jun 1938, *Weatherby, Weatherby s.n.* (MICH). **MAINE**. **Grand Lake.:** Blue Grass Brook, near Camp 11, 5 Aug 1951, *Rolueau 2206* (MICH). **Hancock Co.:** Central Tract, Brooklin, 2 Aug 1914, *Hill 1769* (GH). **Lincoln Co.:** Cathedrdal Woods, Monhegan Island, 29 Jun 1919, *Jenney, Churchill, Hill 3170* (GH). **Piscataquis Co.:** Squaw Moosehead Station, 9 Jul 1917, *Sanford 60605* (GH). **Ostesgo Co.:** N side Old State R d . (F38) ca. 13.5 Km E of Otsego Lake , SE 1/4 sect. 10, T29N R2W. Lat. & Long. 44° 54' 55" N, 84° 31' 46" W, 15 Jul 2002, *A.A. Reznicek 11367* (MICH). **Piscataquis Co.:** Greenville Junction, 8 Jul 1917, *Sanford 6032* (GH). **Somerset Co.:** Jewett Brook Bog, NE of Jewett Pond. 150 feet NW of Jewett Brook and past the turn to Jewett Pond campsites, 4.7 mi NW of Cross Rd. along Rowe Pond Rd., Pleasant Ridge Twp. 0420195E 4998596N UTM Zone 19T (Units: km, Datum: NAD 83), 7 Jul 2002, *Kirschbaum s.n.* (n/a); Pleasant Ridge, Upper Jewett Bog, 5 Jul 1906, *Murdoch 2022* (GH). **Washington Co.:** salt marsh along Sandy R, behind Sandy R Beach, 3.5 mi ENE Jonesport, E side of Hwy 187, 067°32'W, 44°34'N, 7 Jul 1993, *Reznicek 9615* (MICH). **MICHIGAN. Baraga Co.:** along US Highway 41 near W end of Lake Michigamme, 22 Jun 1950, *Richards 3184* (MICH). **Berrien Co.:** Buchanan Bog, 30 May 1930, *Herbert s.n.* (MICH). **Iron Co.:** *Larix* Bog, near Deer Lake, 10 m NE of Crystal Falls, 088°20'W, 46°24'N, 8 Aug 1934, *Grassl 8057* (MICH). **Lake Co.:** Ca. 2.5 mi N of Bristol, 18 Jul 1973, *Voss 14227* (MICH). **Schoolcraft Co.:** T42N, R16W, Sect. 11 NW1/4NE1/4, SW1/4SE1/4, 2 Sep 1971, *Henson 269* (MICH). **Washtenaw Co.:** Gorman Lake Bog on the S end of Gorman Lake off Lindley Rd. in Waterloo State Recreation Area, ca. 8 mi NW of Dexter, 21 Sep 2002, *Kirschbaum s.n.* (n/a). **MINNESOTA. Aitkin Co.:** 0.7 mi S of McGrath, 25 Jun 1940, *Moore, Moore 13263* (MT). **Cook Co.:** S of Grand Portage along Highway 61, 15 Aug 1987, *Castaner 9986* (MICH). **NEW HAMPSHIRE. Coos Co.:** Magalloway River, 3 Aug 1914, *Pease 16207* (GH); Bray Hill Bog, NE of Whitefield of county road 116 (Jefferson Rd.) on the NW side of Bray Hill Rd., Whitefield twp., 0297240E 4920187N UTM Zone 19T (Units: km, Datum: NAD 83), 7 Jul 2002, *Kirschbaum s.n.* (n/a). **Cumberland Co.:** Bog Pond, 0.75 km off Haskell Hill Rd. by way of power line right-of-way on private property, northern Harrison twp., 0370429E 4886298N UTM Zone 19T (Units: km, Datum: NAD 83), 7 Jul 2002, *Kirschbaum s.n.* (n/a). **Hillsborough Co.:** off hiking trail on the W side of Mud Pond in Fox State Forest. Hillsborough twp., 0264272E 4780594N UTM Zone 19T (Units: km, Datum: NAD 83), 4 Jul 2002, *Kirschbaum s.n.* (n/a). **Strafford Co.:** N of Scruton Pond, on Scruton Pond Rd., Barrington twp., 4 Jul 2002, *Kirschbaum s.n.* (n/a). **NEW YORK. Allegany Co.:** Moss Lake Bog on Moss Lake off Sand Hill Rd. ca. 2.5–3.0 mi SW of Houghton, Caneadea twp. 0731623E 469767N UTM Zone 19T (Units: km, Datum: NAD 83), 11 Jul 2002, *Kirschbaum s.n.* (n/a). **Hamilton Co.:** border of The Plains, South Branch of the Moose River, 10 Aug 1950, *Smith, Weaver 7577* (DAO). **Oneida Co.:** *Knieskern s.n.* (MICH). County unknown, Adirondack Mountains, 20 Jun 1936, *Killip 31814* (MICH). **OHIO. Stark Co.:** thickets in center of Brewster Bog, 0.2 mi SE, jct. of M Eaton St and Wellbrook Ave, Navarre Quad, 10 Jul 1984, *Cusick, Denny, Munch 23633* (MICH). **PENNSYVANIA. Elk Co.:** ca. 11 km S of town, 078°15'W, 41°15'N, 18 Jun 1997, *Grund 1918* (MICH). **Tioga Co.:** 4.5 mi NW of Morris, 077°20'51"W, 41°37'07"N, 29 May 1975, *Rothrock 309* (MICH). **VERMONT. Franklin Co.:** Berkshire, 15 Jun 1912, *Underwood 2122* (GH). **WISCONSIN. Jackson Co.:** Bear Bluff Twp, in the old bed of Glacial Lake, T21N, R01E, Sect. 29, 19 Jun 1958, *Hartley 3969* (DAO). **Iron Co.:** between Hwy 51 and Rice L, near CNW RR, 2 mi NW of Mercer, T43N, R03E, Sect. 26 NW1/4, 3 Jul 1976, *Cochrane, Cochrane 7547* (MICH). **WEST VIRGINIA. Giles Co.:** Big Good Bed at head waters of Little Stony Creek, Salt Pond Mt., 9 Jun 1946, *Wood 5972* (GH). **Pocahontas Co.:** 0.2 mi S of park office, Droop Mountain Battlefield State Park, NW, US Rte 219, N of Droop, 1 Jun 1990, *Cusick 28918* (MICH); ultimate headwaters of First Fork of Shavers Fork Creek, ca. 2 air mi 110 degrees from Shavers Fork crossing at Randolph County line, ca. 4.2 air mi NE of Cass Scenic RR State Park off For Serv Rd 235, Back Allegheny Mtn, Monongahela National Forest, 27 Jul 1994, *Nelson 15881* (MICH). **Tucker Co.:** beyond Little Blackwater, near camp 70 near 3200f, Canaan Valley, 1 Aug 2007, *Allard 10106* (GH).

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## REFERENCES

Abbott, L.A., F.A. Bisby, and D.J. Rogers. 1985. Taxonomic analysis in biology: computer, models, and databases. Columbia University Press, New York, NY.

Anderson, D.S., R.B. Davis, S.C. Rooney, and C.S. Campbell. 1996. The ecology of sedges (Cyperaceae) in Maine peatlands. Bull. Torrey Bot. Club. 123:100–110.

Applied BioSystems. 2000. AFLP Plant Mapping (#4303146). Applied BioSystems, Foster City, CA.

Bernard, J.M., D. Solander, and J. Kvet. 1988. Production and nutrient dynamics in *Carex* wetlands. Aquatic Bot. 30:125–147.

Berres, M.E. 2002. "Studies in Avian Genetic Population Structure." University of Wisconsin Madison, http://ravel.zoology.wisc.edu/sgaap/AFLP_html/AFLP.htm. (accessed September 2003).

Boiven, B. 1967. Énumération des plantes du Canada. Naturaliste Canad. 94:523.

Braverstock, P.R. and C. Moritz. 1996. Project design. In: D.M. Hillis, C. Moritz, and B.K. Mable, eds., Molecular systematics, 2nd edition. Sinauer Assoc. Inc., Massachusetts. Pp. 249–320.

Brown, J.H. and M.V. Lomolino. 1998. Biogeography, 2nd edition. Sinauer Associates, Inc., Sunderland, MA.

Felsenstein, J. 2004. PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Gleason, H.A. and A. Cronquist. 1991. Manual of vascular plants of northeastern United States and Adjacent Canada. Princeton, NJ.

Hipp A.L., A.A. Reznicek, P.E. Rothrock, and J.A. Weber. 2006. Phylogeny and classification of *Carex* Section *Ovales* (Cyperaceae). Int. J. Plant Sci. 167:1029–1048

Hipp, A.L. 2004. Molecular Systematics of *Carex* section *Ovales* (Cyperaceae). Ph.D. dissertation. Univ. of Wisconsin, Madison.

Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. B. Soc. Vaud. Sci. Nat. 44:223–270.

Kardolus J.P., H.J. van Eck, and R.G. van den Berg. 1998. The potential of AFLPs in biosystematics: a first application in *Solanum* taxonomy (Solanaceae). Pl. Syst. Evol. 210:87–103.

Kirschbaum, C.D. 2005. Taxonomy of *Carex trisperma* varieties (Cyperaceae) based on morphology, phytogeography, ecology, and Amplified Fragment Length Polymorphisms (AFLPs). M.S. thesis. Eastern Michigan Univ., Ypsilanti.

Knight, O.W. 1906. A new variety of *Carex trisperma*. Rhodora 8:185

Landry, P.A. and F.J. Lapointe. 1996. RAPD problems in phylogenetics. Zool. Scr. 25:283–290.

Lessa, E. P. 1990. Multidimensional analysis of geographic genetic structure. Syst. Zool. 39:242–252.

McCune, B. and M.J. Mefford. 1997. PC-ORD: Multivariate Analysis of Ecological Data, Version 3.0. MjM Software Design, Gleneden Beach, OR.

Nei, M. and W.H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA. 76:5269–5273.

Riseberg, L.H., T.E. Wood, and E.J. Baack. 2006. The nature of plant species. Nature 440:524-527.

Sokal, R.R. and P.H. Sneath. 1963. Principles of numerical Taxonomy. W.H. Freeman and Company, San Francisco, CA.

Sprengel, C.P.J. 1826. Systema Vegetabilium, edito decimal sexta 3:809. Gottingae, sumtibus Librariae Dieterichianae.

Toivonen, H. 2002. *Carex* Linnaeus sect. *Glareosae* G Don. In: Flora of North America Editorial Committee, eds., Flora of North America. Vol. 23, Magnoliophyta: Commelinidae (in part): Cyperaceae. Oxford Univ. Press, Oxford and New York. Pp. 311–321.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407–4414.